VARIATION IN PEPPER PUNGENCY AS A FACTOR IN THE QUALITY OF PROCESS CHEESE WITH JALAPENO PEPPERS

by

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B.S., Kansas State University, 1971

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1979

Approved by:

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ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. James T. Marshall, my supervising professor, for his valuable guidance, patience, and assistance during the course of my graduate studies. Appreciation is also extended to Dr. Richard Bassette and Dr. B. E. Brent for their valuable help and assistance while serving as members of my graduate committee.

In addition, I would like to express appreciation to Dr. Arthur Dayton for his help with the statistical analysis used in this study and to Don Sapienza for his valuable help with the gas chromatographic studies.

I would also like to thank the Animal Science and Industry Department for financial support in the form of a graduate assistantship.

A special thank you is also extended to my wife, Cathy, for her help in typing my thesis and for her encouragement during my graduate studies.

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Manhattan, Kansas

January, 1979

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INTRODUCTION

Since the pungency of hot peppers determines to a great extent their quality and the price that will be paid for them, there has long been an interest in developing accurate methods of analyzing pungency. Manufacturers of process cheese with jalapeno peppers share this interest as variations in the pungency of the peppers they buy may affect the acceptability of their product by consumers in the marketplace.

Pungency, for purposes of this study shall be defined as the burning sensation in the mouth and throat commonly associated with hot peppers. A review of literature indicated that the pungency of peppers may be affected by such factors as, variety of pepper, location of cultivation, type of processing and preservation used, as well as stage of ripeness and season of harvest (18, 24, 26).

For manufacturers of process cheese with jalapeno peppers, the question also exists as to what extent variations in the pungency of peppers used in their product are perceivable by their customers. In short, is it necessary to analyze each lot of peppers purchased for pungency and adjust the amount used in the cheese accordingly? In addition, what range of pungency might be expected in lots of jalapeno peppers purchased from different suppliers or locations?

The purpose of this study was to examine some of the questions stated in the preceding paragraph. Briefly stated, the objectives were:

1) To examine the accuracy of several methods of pungency determination and potential for use by food processors.
2) To determine what variation in pungency might be expected in a cross section of jalapeno peppers obtained from a variety of suppliers.
3) To determine to what extent variations in pungency of peppers are detectable when used in pasteurized process cheese with jalapeno peppers.

REVIEW OF LITERATURE

CAPSICUMS

Capsicum peppers were originally cultivated by Indians in South America but by 1660 cultivation began to spread throughout the world. As a result of the selective breeding programs and introduction of capsicums into new geographical regions with varying climates, many new varieties came into existence. Today capsicums vary widely in size, shape, color, flavor, and pungency (hotness).

The jalapeno pepper is one of a number of varieties of the Capsicum pepper species, Capsicum annuum (18). All capsicums belong to the plant family Solanaceae which also includes the egg plant and tomato (18). To date, over 200 varieties of capsicums have been described (18) but according to Maga (26) only five species are currently recognized: Capsicum annuum, Capsicum frutescens, Capsicum chinense, Capsicum pendulum, and Capsicum pubescens.

The name capsicum is believed to have been derived from one of two sources: the Greek term "kapso" meaning "to bite", or the Latin word "Capsa" for box, possibly referring to the internally partitioned fruit pod characteristic of these peppers (26).

Capsicum annuum is the most widely cultivated of the capsicum species making up almost all of the U.S. and European production (26). In addition to the jalapeno, Capsicum annuum, also includes most

sweet pepper varieties, paprika, and cayenne pepper (15, 26).

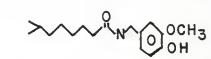
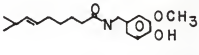
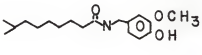
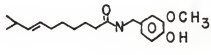
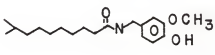
Anatomically, the structure of the jalapeno pepper is similar to other peppers of the genus Capsicum. Generally there is an outer wall enclosing two to four interlocular septae and a central core to which numerous small seeds are attached (18).

CAPSAICIN

The burning or pungent sensation which is characteristic of hot peppers is believed to result from the reaction of the chemical capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) and four closely related compounds on the pain and touch receptors of the mouth and throat (26, 51). In addition to capsaicin, Bennett and Kirby (3) using mass spectrometry in 1968 reported the existence of nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin. By comparing the results of gas-liquid chromatography and organoleptic studies, Todd et al. (51) were able to separate and determine the relative pungency of each of the five pungent compounds found in Capsicum annuum. The name, chemical structure, and reported threshold pungency of each of these compounds is shown in Table 1.

Capsaicin is a fat soluble, odorless, and colorless compound. It was first isolated in 1876 by Tresh (52) and described structurally by Nelson in 1920 (32).

Table 1. Natural capsaicinoids.

Name	Structure	Threshold Pungency (millions)[1]
Nordihydrocapsaicin		9.3
Capsaicin		16.1
Dihydrocapsaicin		16.1
Homocapsaicin		8.9
Homodihydrocapsaicin		8.1

From Todd, P. H. Jr., Bensinger, M. G., Biftu, T. 1977. Determination of pungency due to capsicum by gas-liquid chromatography. Journal of Food Science 42(3): 660-665.

[1] Greatest dilution of capsaicinoid in 3% dextrose solution in which taste panel members were able to detect pungency.

Studies have shown that double bonds which may be present in the acid portion of the pungent molecules have no effect on hotness. However, varying the length of the acid chain has a great effect (26). In 1925 Jones and Pyman (19) noted that the loss of the phenolic hydroxy group eliminated pungency while the addition of a second phenolic hydroxy group increased pungency. Oxidation of pungent compounds with potassium permanganate or dichromate has been shown to destroy the pungent effect (15).

In pure form, capsaicin forms white, pearly leaflets melting sharply at 64-65° C. Capsaicin is almost insoluble in water but readily soluble in methanol, ethanol, acetone, chloroform, ethyl acetate and ether and moderately soluble in "hot light petroleum" (boiling range 60-80° C) (15).

Capsaicin is used in medicine as a carminative (skin irritant) and rebefacient (stomach gas reducer) and in foods as a spice (15).

VARIATIONS IN PUNGENCY OF PEPPERS

While the jalapeno pepper (Capsicum annuum) is sometimes noted for its hotness, capsaicin content varies greatly and is generally less than .2% of the dry weight (40). Several other capsicum species and varieties, generally producing smaller fruits, (Capsicum frutescens, Capsicum annuum (variety minimum) have been shown to be considerably more pungent with capsaicin levels of up to 1.0% (40).

Weisenfelder et al. (58) reported an average capsaicin content of 0.113% in 10 experimental and 2 commercial jalapeno varieties grown in Texas. In contrast, Karawya et al. (20) reported an average of 0.748% capsaicin in five samples of Capsicum minimum and 0.420% capsaicin in five samples of Capsicum frutescens.

Geographical location has also been shown to effect the pungencies of capsicums. As shown in Table 2, Berry and Samways (4) found as much as a twofold difference in pungency among identical species of capsicum grown in different geographical locations.

In addition, season of the year and stage of maturity have been shown to influence capsaicin levels in capsicums. Balbaa et al. (2) determined in their study that pungency could be detected at an earlier stage of maturity in autumn plants than in summer plants, although the summer plants would attain higher final pungency levels.

Finally, the distribution of the pungent compounds within the fruit does not appear to be uniform. Tandon et al. (47) reported that the pericarp which comprises 40% of the pepper contains 89% of the capsaicin and that the seeds which make up 54% of the pepper contained 11% of the capsaicin. Balbaa et al. (2) further reported that most of the capsaicin was located in the dissepiment portion of the fruit with the ratio of capsaicin in the dissepiment and pericarp portions to be 100:6, respectively. Both Balbaa (2) and Maga (26) expressed doubt that the seeds actually contained capsaicin. Rather, they felt that the

Table 2. Influence of geography and botanical source on pungency.

Geographical Source	Botanical Source	Pungency[1]
Bombay	Capsicum annuum	30
Natal	Capsicum annuum	60
Japan	Capsicum annuum	40
Nyassaland	Capsicum annuum	60
India	Capsicum annuum	50
Sierra Leone	Capsicum minimum	150
Sudan	Capsicum minimum	100
Nombassa	Capsicum minimum	80
Mozambique	Capsicum minimum	90
Zanzibar	Capsicum minimum	110
Sudan	Capsicum minimum	140

From Berry, H. and Samways, E. K., The pungency values of capsicum and tincture of capsicum, (1937) Q. Rev. Pharm, Pharmacol., 10:387.

[1] Twice the reciprocal of dilution found to be pungent.

findings of earlier researchers indicating the presence of capsaicin in the seeds resulted from surface contact contamination of the seeds during separation from the remainder of the fruit. Huffman et al. (18) concluded after gas chromatographic studies that most of the pungency can be found in the cross wall portions of the pepper with limited amounts in the placental areas. Huffman's work verified the earlier studies of Prokhava and Prozorovskaya (37) using colorimetry. The presence of blisters or pustules containing capsaicin on the cross wall portion of peppers was noted by Villalon (56) in 1976.

Curiously, Huffman et al. (18) found much higher capsaicin levels in peppers which had been thermally processed than in raw peppers. In addition, they found the capsaicin to be more evenly distributed in the processed peppers. They theorized that these changes resulted from the volitilization of capsaicin and the lysing of cells which allowed the capsaicin to spread more evenly throughout the fruit. They further proposed that complexing agents may have been split off leaving the capsaicin free and more readily available for analysis.

The temperature used to dry peppers has also been shown to influence the pungency. Lease and Lease (23) determined that peppers dried at a temperature of 150^{o} F has a significantly higher pungency than peppers dried at higher and lower temperatures. Their results verified the earlier findings of Van Arsdel et al. (54).

The pungency of capsicum extracts is dependent on the length of extraction. Berry and Samways (4) determined that relatively long extraction times were necessary, concluding that 6 hours was satisfactory.

ANALYSIS OF CAPSAICIN

Since the pungency of red peppers determines to a great extent the price that is paid for them, there has long been an interest in a reliable method of evaluating capsaicin levels in peppers and pepper oleoresins.

Some of the first methods developed for capsaicin analysis were organoleptic tests. Trained taste panel members were employed to test a series of diluted pepper extracts containing capsaicin and evaluate each for hotness. The level of dilution at which the panel members could first perceive the burning sensation associated with hot peppers was used to compute the pungency value for each sample.

The oldest of these methods was first developed by Scoville (44) in 1912 and is still recognized as the official method of the American Spice Trade Association. A later method, developed by Berry and Samways (4), which also depends on the detection of threshold pungency, is now the official method of the British Pharmaceutical Codex.

As reported by Heath (15) and Maga (26), the accuracy of organoleptic methods for pungency is low because sensitivity to capsaicin can vary between individuals and can be effected by colds, temperature of the sample, taster fatigue, etc. However, pungency methods may have an advantage over some other organoleptic measurements in that they depend on the detection of threshold pungency rather than an olfactory determination.

Numerous chemical analytical methods for capsaicin have been developed although the search for an accurate and simple one goes on. Most modern methods require some type of sample preparation or separation prior to the actual determination although some of the earlier methods did not.

One of the earliest instrumental methods, developed by Von Fodor (57) and later modified by Tice (49), used a colorimeter to measure a blue color produced by the interaction of the hydroxyl group of capsaicin with vanadium oxytrichloride. This method was shown by Hayden and Jorden (13) to be inaccurate due to interferences by other components giving the same color reaction.

Norgrady (34) developed a fluorometric method which involved the titration of a uv-irradiated capsicum extract with an alcoholic solution of picric acid until fluorescence disappeared. The resulting value for capsaicin plus coloring material present could be compared

to the value obtained from titrating a solution of natural capsaicin dyes of the same intensity as the sample, thus estimating the actual capsaicin content.

A number of other colorimetric methods have also been developed. A method using Folin-Denis reagent to produce a blue color and comparing the color produced by capsicum extracts to standard solutions of vanillin, which reacts similarly, was developed by North (35) in 1949. Schenk (41) used ammonium vanadate and hydrochloric acid to produce a blue-green color in the decolorized eluate resulting from a chromatographic separation. Other methods employing chromatographic separation have been developed by Fujita et al. (9) and Schulte and Kruger (42). Fujita's chromatographic separation was followed by the colorimetric determination of capsaicin using the reduction of molybdophosphoric acid by capsaicin in an alkaline solution as developed by Buecki and Hippenmeier (5). Schulte and Kruger measured the color produced by the coupling of diazobenzenesulphonic acid with capsaicin.

In a study published in 1959 (15) a Joint Committee of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry directed by H. B. Heath evaluated all of the colorimetric methods previously outlined. As a result of this study Heath reported that none of the methods were completely reliable but suggested the method of Schulte and Kruger as the one most suitable for routine use.

Heath noted, however, that the azo dye formed in this procedure could be produced by any amine or phenol with an unoccupied ortho or para position and thus was not specific for capsaicin. He further noted difficulties in handling potentially explosive dry diazonium salts and the troublesome necessity of preparing a standard graph using reference samples of pure capsaicin each time a determination was made. For separation of capsaicin the committee recommended either an ether-alkali extraction series or separation by a column consisting of a mixture of aluminum oxide and activated carbon.

In a second study published in 1964 (16), the same committee examined the colorimetric method of Holo et al. (17) using Gibbs Reagent (2, 6 Dichlor-p-benzo-quinone-4-chloroimine) to produce a colored complex with capsaicin. While the committee reported difficulties with the solvents used to dilute the reagent and some reproducibility problems resulting from reagent instability, they determined the method to be the best of the colorimetric procedures they had reviewed.

In both studies, the committee also suggested the use of an ultra-violet spectrophotometric method utilizing absorbances obtained from methanolic capsaicin sclutions at wavelengths of 248 nm and 296 nm.

Suzuki et al. (46) developed a method of determining capsaicin by using an ultra-violet spectrophotometer to make direct readings

of samples separated by column chromatography.

Thin-layer chromatography has been successfully used by several researchers (6, 8, 31, 38) to separate capsaicin and some of its individual components. A variety of coatings and solvent systems were employed in these studies. However, Salazar (40), in a recent study of methods used to determine capsaicin, reported that thin-layer methods were not effective with highly colored samples.

Separation of capsaicin by paper chromatography was attempted by Kosuge et al. (22) who were able to attain only partial separation. More recently, however, Govendorajan and Ananthakrishna (10) reported good success in separating capsaicin by a paper chromatographic method and quantifying the results using the previously mentioned Gibb's reagent.

Several researchers have attempted to use gas-liquid chromatography (GLC) as an analytical method to separate capsaicin and its analogues. Studies by Morisson (29), Holo et al. (17), Di Cecco (7), and Hartman (12) estimated only the gross capsaicin content of the pepper sample and did not attempt to isolate the individual pungent compounds. However, Masada et al. (27), by using a combination of GLC and mass spectrometry were able to successfully separate and identify all capsaicin analogues except homocapsaicin. Recently Todd et al. (51) developed a GLC method for identification of

all five naturally occurring capsaicinoids as well as several synthetic derivatives obtained from raw capsicums, oleoresins, tinctures, and plasters.

PROCESS CHEESE

Process cheese has been defined by Van Slyke (55) as a food made from several lots of cheese that are ground and mixed together by stirring or heating. Water, seasoning, color and emulsifying salts may be added. Flavoring materials like pimentos and spices are sometimes used, or cheese may be smoked or flavored with smoke condensate for additional character.

Process cheese originated in Europe in the late 1800's with the first patent reportedly issued in England in 1899 (21). The process cheese industry in the United States began in 1916 when J. L. Kraft was issued a U. S. patent for a process involving the heating of natural Cheddar cheese with alkaline salts used as emulsifying agents. Recently, about 39% of the per capita consumption of cheese in the United States was process cheese and related products (25). According to Meyer (28), Cheddar cheese is the cheese most commonly used for processing throughout the world.

Thomas (48) lists the following advantages of process cheese over natural cheese:

1) Reduced need for refrigeration in storage and transit.

2) The flavor and body of process cheese does not alter during storage.

3) Process cheese can be flavored to suit taste.

4) Process cheese can be easily packaged in small containers more attractive to consumers.

The manufacture of process cheese generally begins with the careful blending of two or more lots of aged and young cheese. The exact proportions used depends on the texture and flavor of the cheese used and the specifications of the finished product. Generally, too much young cheese will produce an excessively mild taste and a body which is very hard. On the other hand, excessive amounts of aged cheese will often result in a very sharp taste or possibly even strong off-flavors with a soft body and poor emulsification. According to Thomas (48) the source of the most flavor in process cheese is the proportion and maturity of old cheese in the blend, while the young cheese determines the body characteristics.

To prevent the separation of the cheese into its three main components: fat, protein, and water, during processing, emulsifying salts are used. In addition, emulsifiers also function to regulate pH and create a desirable short body texture by reducing the size of the paracaseinate molecule. Three general types of emulsifying salts are commonly used: citrates, monophosphates, and polyphosphates. Each emulsifier has its own characteristics and the salt or combination of

salts used will vary according to cost and desired effects. According to Kosikowski (21) sodium citrate has the "best general all around qualities".

To improve such factors as appearance, flavor, texture, and keeping quality, various types of optional ingredients may be added to certain process cheese products. These include: whey, meat products, fruits, vegetables, preservatives, and coloring (53).

The jalapeno pepper is an example of a vegetable (spice) commonly used in the process cheese industry. Conversations with several manufacturers of process cheese with jalapeno peppers (29, 45, 50) revealed that the amount of peppers added to the blend will vary according to the geographical location in which the cheese is to be sold. Their formulations varied from 2 to 15 percent pepper with the higher concentrations being sold in Southern and Western areas while milder cheese was preferred by Eastern and Northern consumers.

Meyer (28) states that the addition of spices may have an effect on pasteurization and sterilization and also suggested the possible advantages of using seasonings as a means of eliminating off flavors in process cheese. Reddy (39) determined that moderately rancid Edam cheese can be used to produce an acceptable process cheese if combined with an equal part of young Cheddar and 10% jalapeno peppers.

MATERIALS AND METHODS

For this study, eight samples of jalapeno peppers were obtained. The samples were obtained from suppliers in several geographical locations who utilized a variety of methods for processing and preservation (Table 3). Both fresh and processed (whole or diced) jalapeno peppers were used in this study, with the latter being either dryed, pickled, or blanched and frozen.

A representative portion of each pepper sample was dried and analyzed for capsaicin by each of three different methods: an organoleptic test, a uv-spectrophotometric determination following a column separation, and a gas-liquid chromatographic (GLC) analysis.

Peppers from several sources were utilized in the manufacture of pasteurized process cheese with jalapeno peppers. Cheddar cheese used to formulate the process cheese was obtained from three lots in storage at the Kansas State University Dairy Plant.

Each cheese lot was tested for moisture, fat, and pH and mixed with a measured amount of the jalapeno pepper being tested. The jalapeno peppers were blended for 3 minutes in a Waring blender prior to their incorporation in the process cheese. Peppers obtained in the dry form (Table 3) were rehydrated (3 parts pepper to 7 parts water) prior to blending (Table 4). Blending was necessary so as to minimize differences in the appearance of the process cheese submitted to taste panels.

Table 3. List of peppers and sources.

Sample No.	Description	Method of Processing and Preservation	Source and Date Obtained
1	Whole, Fresh	Refrigeration = 5 C	Safeway Store Village Plaza Shopping Center Manhattan, Kansas 66502 6/20/78
2	Whole, Fresh	Refrigeration = 5 C	Dillon's Food Store West Loop Shopping Center Manhattan, Kansas 66502 6/21/78
3	Whole, Fresh	Refrigeration = 5 C	Road Side Market East Highway 24 Manhattan, Kansas 66502 9/15/78
4	Whole, Fresh	Refrigeration = 5 C	Safeway Store Village Plaza Shopping Center Manhattan, Kansas 66502 6/20/78
5	Whole, Frozen	Blanched in boiling 3-4% salt brine for 60-90 seconds, cooled, then frozen	Sekan Cheese Co. Girard, Kansas 66743 8/22/78
6	Whole, Canned	Pickled. Packed in #10 can with vinegar, salt onions, sesame oil, carrots, pickles, dehydrated garlic and spices	Clemente Jacques Y Cia S. A. De C. V. Libramiento F. C. Cintura No. 1 Mexico City 1, D. F. 7/6/78
7	Diced, Pickled	Pickled in salt and fermented 2-3 weeks, cured 3 months. Packed in acidified brine of pH 3.4 - 3.5 in 25 lb. plastic pail	Pikle - Rite Co. Pulaski, Wisconsin 54162 8/22/78
8	Diced, Dried	Dried on trays to 8% moisture in hot air tunnels (145 F - 175 F)	Santa Maria Chili Inc. P. O. Box 1028 Santa Maria, California 93456 7/6/78

A five member taste panel consisting of 3 students and 2 faculty members at Kansas State University evaluated each batch of jalapeno cheese on the basis of pungency (hotness).

The manufacture of process cheese with jalapeno peppers and all testing of cheese and pepper samples was conducted using the equipment and laboratory facilities of the Animal Science and Industry Department of Kansas State University and the Kansas State University Dairy Plant.

ANALYSIS OF PUNGENCY

To determine the pungency of each pepper sample, three types of determinations were used. Diagram 1 is a flow chart illustrating the major steps of each determination.

DRYING

Prior to each pungency determination it was necessary to dry a portion of each pepper sample to approximately 10% moisture. This was accomplished by placing the peppers in an oven maintained at 60-65 C for a period of twelve hours, in the case of whole samples, and six hours for diced samples. These drying times and temperatures were determined by Lease and Lease (24) to achieve the desired moisture while retaining maximum pungency. Following the recommendations of the American Spice Trade Association (ASTA),

Diagram 1. Outline of analysis for pungency and capsaicin in jalapeno peppers.

Dry peppers at 60 C to / 10% moisture.
Grind the dried peppers to pass 30 mesh sieve.

Scoville Pungency (Sensory)	Capsaicin (U. V. Absorbance)	Capsaicin (GLC)
1. Extract in 95% ethanol.	1. Extract using 1% methanol in ethyl acetate.	1. Extract with methanol.
2. Prepare series of dilutions in 3% dextrose.	2. Pass through absorption column. [1]	2. Concentrate to 1-2 mg capsaicin per ml.
3. Taste panel tests dilutions for pungency.	3. Wash interferences from column with 1% methanol in ethyl acetate.	3. Mix tincture with BSTFA[2] and THF[3].
4. Pungency calculated in Scoville units on basis of greatest dilution found to be pungent by taste panelists.	4. Elute capsaicin with absolute methanol.	4. Inject .5 ul into gas chromatograph.[4]
	5. Determine the absorbance of eluate at 280 nm with U. V. Spectrophotometer.	5. Determine % capsaicin in sample by comparing to standard samples prepared from pure capsaicin.
	6. Determine amount of capsaicin in sample by comparing absorbance to standard curve obtained from pure capsaicin.	

1 Column contains acidic and basic alumina, charcoal, nyflo-supercell, and anhydrous sodium sulfate.

2 N, O-bis-(trimethylsilyl)-trifluoreacetamide.

3 Tetrahydrofuran.

4 GLC column: glass 2m x .085 in. inside diameter packed with 3% SE-30 on chromasorb GHP, 100-120 mesh.

method 1.0, dried samples were ground to pass through a 30 mesh (ASTA 1.0 specifies 40 mesh) standard screen, sealed in jars, and stored in a refrigerator until needed for pungency analysis (36).

PUNGENCY ANALYSIS - ORGANOLEPTIC

Organoleptic pungency (Scoville value) of pepper samples was determined using the Scoville Heat Test of the American Spice Trade Association (ASTA), method 21.0 (36), and the U.S. Quartermaster Corps (1) with modifications as suggested by Suzuki et al. (46) and Hart and Fischer (11).

The modified procedure entailed placing one gram of dried, ground pepper in 50 ml of 95% ethanol and extracting for 24 hr at room temperature with occasional shaking. After extracting, the solution was filtered using No. 1 Whatman filter paper and the filtrate was placed in a stoppered flask. Five ml of the filtrate was transfered into a 100 ml volumetric flask which was then filled to the 100 ml mark with 3% Dextrose (46) in distilled water to produce a 1 to 1000 dilution of the dried pepper extract. Into a series of 50 ml volumetric flasks 40, 20, 10, 5, 3.75, 2.5 and 1.87 ml portions of the 1:1000 dilution were successively transfered and diluted to the 50 ml mark with 3% Dextrose. The resulting dilutions were 1 to: 1,250, 2,500, 5,000, 10,000, 15,000, 20,000 and 30,000 respectively.

The pungency (Scoville) value was determined as recommended by Hart and Fischer (11) by calculating the average for the dilutions at which the panel members first reported pungency, discarding any results obviously out of range.

Taste panelists were supplied with four or five numbered sample cups which contained 5 ml portions of a series of dilutions appropriate in concentration for the pungency of the sample being tested. The appropriate dilution range was selected by preliminary taste testing as the dilutions were being prepared. A properly prepared series of dilutions would begin with a dilution below the expected threshold pungency concentration for the panel, and would include dilutions of such concentration as to be well above the expected threshold, i.e., the expected threshold was bracketed with dilutions. Panelists were instructed to taste each dilution, beginning with the most dilute (cup no. 1) and proceeding through the succeeding dilutions until the first definite sensation of pungency (burning in mouth and throat) was perceived. The number of the cup containing the pungent dilution was then recorded on the form provided (See Appendix, page 62).

The 5 ml portion was to be tossed onto the back of the throat and swallowed at once. A minimum time of 5 minutes was allowed between samples with the panel member instructed to sip warm water between each dilution.

CAPSAICIN DETERMINATION BY COLUMN CHROMATOGRAPHY FOLLOWED BY UV SPECTROPHOTOMETRY

Capsaicin concentration was determined using the uv absorbance method developed by Suzuki et al. (46). A two gram sample of dried and ground jalapeno peppers was placed in 50 ml of 1% methanol in ethyl acetate and refluxed for 6 hours in a Goldfisch extractor, before filtering through Whatman No. 1 filter paper. The filtrate was passed through 1.75 cm x 50.0 cm glass chromatographic column packed with successive layers of: 2 g anhydrous sodium sulfate, 2 g 1:1 mixture of Norit-A acid washed charcoal and hyflo-supercel, 2 g acidic alumina, and 12 g basic alumina. To remove interfering substances, 300 ml of 1% methanol in ethyl acetate was passed through the column instead of the 250 ml recommended by Suzuki et al. (46). Capsaicin was then eluted using 300 ml of methanol. The absorbance from an aliquot of the 300 ml eluate was then measured at 280 nm using a Gilford Model 240 uv spectrophotometer. The capsaicin content in the sample was then calculated from a standard curve previously prepared using pure capsaicin obtained from the Sigma Chemical Co., St. Louis, Missouri.

Early trials produced eluates with abnormally high absorbances and poor reproducibility. This difficulty was overcome by passing approximately 4 l of ethyl acetate through the column for additional purification.

The standard curve was obtained using a Wang 700 series programable calculator programed for quadratic regression analysis. Concentrations of unknown samples were then obtained from their absorbance readings using the previously determined standard curve parameters.

CAPSAICIN DETERMINATION BY GAS-LIQUID CHROMATOGRAPHY (GLC)

The GLC method developed by Todd et al. (51) was used to determine the capsaicin content of each of eight pepper samples.

Tinctures (extracts) of each pepper sample were prepared by extracting a carefully weighed amount (about 5 grams) of dried pepper for 12 hours in 50 ml methanol using a Goldfisch extractor. Sample volume was then reduced to about 10 ml using a rotary evaporator at a temperature of 55° C. Final volume reduction to 5 ml was accomplished by placing the solution in a 55° C water bath and bubbling carbon dioxide (CO_2) gas through the solution until the volume was small enough to be placed in 5 ml volumetric flask. Methanol was used to increase sample volume to the 5 ml mark on the flask. The reduction of sample volume to 5 ml was intended to produce the recommended capsaicinoid concentration of 1-2 mg/ml. This calculation was based on the report by Weisenfelder et al. (58) of an average capsaicin concentration of 1.12 mg/g of dry jalapeno peppers.

For GLC analysis, 2 ml of the sample tincture was combined with 2 ml of tetrahydrofuran (THF) solvent. From this mixture, .5 ml was transferred to a dry 1 ml serum bottle and mixed with 50 ul of N, O-bis(trimethylsilyl)-trifluoracetamide (BSTFA) (Pierce Chemical Company, Rockford, Illinois). BSTFA is a silylating agent used to form a derivative with capsaicin which separates from other interfering substances during chromatographic separation (51). Samples were thoroughly mixed and allowed to stand a minimum of 1 hr before injecting .5 ul into the chromatograph.

A Hewlett-Packard Model 5703A dual column gas chromatograph equipped with a Model 7671A autosampler and flame ionization detectors was used for the analysis. A detector temperature of 300° C and an injection port temperature of 200° C was maintained throughout the study. Oven temperature was programed beginning at 200° C for 2 minutes and then increasing at 4° C per minute to 230° C and held for 16 minutes at the peak temperature. A Spectra-Physics Autolab Minigrator was used to integrate the peak areas which were recorded by a Sargent Model SR recorder.

Glass columns measuring 2 m in length with dimensions of 1/8 in outside diameter and .085 in inside diameter were used throughout the analysis. The interior of the tubing was silylanized using a solution of 5% dimethyldichlorosilane in toluene. The tubing was then rinsed with approximately 20 ml portions of toluene and methanol in that order.

Columns were filled with a packing consisting of 3% $_{S}$E-30 on Chromasorb GHP, 100-120 mesh and conditioned overnight at 300° C with carrier gas flow of 10-15 cc/min.

The retention time of the capsaicin peak was determined by comparison to standard samples containing pure capsaicin and a closely related compound n-tetracosane (51) obtained from the Sigma Chemical Co., St. Louis, Missouri.

Capsaicin content of the sample was calculated by comparing the area of the sample peak with the peak areas produced by standard samples prepared from pure capsaicin using the formula below:

$$\%\text{wgt/wgt} = \frac{(\text{Area of Unknown})\ (\text{Wgt Standard})}{(\text{Area of Standard})\ (\text{Wgt Unknown})} \times 100$$

CHEESE USED IN PROCESSING

Three lots of Cheddar cheese were chosen for processing from those in storage at the Kansas State University Dairy Plant. Two lots of aged cheese, stored for 14 and 16 months, respectively, and one lot of young cheese (5 months old) were used to produce the pasteurized process cheese used in this study. Each lot was tested for pH, moisture, and fat.

pH OF CHEESE

The pH of natural and process cheese samples was measured using the standard indicating pH measurement for cheese as described

by Kosikowski (21). After a 2-point standardization with buffers of pH 5 and pH 6 at room temperature, the combination Calomel electrode of a Corning Model 119 pH meter was immersed directly into 10 g of a finely minced cheese sample. The temperature of the sample was taken with a glass thermometer, the meter adjusted to the sample temperature, and the pH measurement observed and recorded. The combination electrode was rinsed with ether and distilled water between each reading.

MOISTURE OF CHEESE

Moisture of cheese samples was determined using the oven method described by Kosikowski (21). Samples of approximately 2 g were placed in previously weighed 5.0 cm x 1.75 cm aluminum sample dishes with covers which had been stored overnight in a dessicator. Samples were then weighed and placed in a 100 C drying oven for 24 hours. After drying, samples were removed and placed in a dessicator and allowed to cool to room temperature before weighing. Moisture content was recorded as a percent of sample weight lost during drying to constant weight.

FAT CONTENT OF CHEESE

Fat content of cheese samples was determined using a modified Babcock fat test (14). Briefly, small strips of cheese were placed

into tared Paley test bottles and combined with 10 ml of distilled water and 15 ml of (Sp. gr. 1.83) sulfuric acid. Bottles were placed in a mechanical shaker for 5 minutes, then centrifuged for 5 minutes. Distilled water was then added in two steps; first to fill the body of the bottle, then the neck, alternating with 2 minute centrifugations. The bottle was transferred to 60 C water bath for a minimum of 5 minutes before measuring and reading the percent fat directly from the sample bottle neck using glymol to facilitate reading.

MANUFACTURE OF PROCESS CHEESE WITH JALAPENO PEPPERS

Equal amounts of aged and young Cheddar cheese were blended with salt (1%), sodium citrate (2%), water, and peppers in the bowl of a KitchenAid Model K5A mixer with a GLAS-COL fiberglass heating mantle placed under the mixing bowl. Prior to adding the ingredients, the mixer and bowl were thoroughly cleaned and sanitized. Melted cheese and added ingredients were heated to a temperature of 74° C for 2 min before being poured into sanitized plastic cottage cheese containers lined with plastic wrap. Containers were then sealed and placed in a refrigerator at 5 C until used in taste panel studies.

Before being used in process cheese, peppers were thoroughly drained of any packing juices and stems were removed. Each lot of peppers was then finely chopped in a Waring blender and weighed before being added to the cheese formulation.

Two series of process cheese were manufactured for taste panel evaluation. Series I utilized peppers obtained from 5 different sources which were formulated at a constant level (c2. 3%, see Table 4). This concentration was selected based upon conversations with several commercial manufacturers of process cheese with jalapeno peppers concerning typical pepper concentrations (29, 45, 50). Series II used peppers obtained from a single source but varied the pepper concentration from 0% to 5%. The various process cheese formulations are in Table 4. Each lot of process cheese was analyzed for moisture, fat, and pH as described above, to ensure that it conformed to federal specifications for such products.

TASTE PANEL EVALUATION

Taste panel evaluations of process cheese samples were conducted in various rooms and offices in Call Hall on the Kansas State University Campus. Generally, the tasting was done in late morning or afternoon as recommended by Larmond (23).

Preliminary tests, using pepper extracts, were conducted to select 5 panelists with good reproducibility and a normal range of sensitivity to pepper pungency as suggested by the American Spice Trade Association (36).

Table 4. Process cheese formulations.

Series I - Different Pepper Samples - Constant (3%) Pepper Concentration

Pepper Sample No.[1]	Grams Pepper	Grams Aged Cheddar (14 months)	Grams Young Cheddar (5 months)	Sodium Citrate (grams)	Salt (grams)	Water (mls)
3	30.0	485	485	20.0	10.0	60
5	30.0	485	485	20.0	10.0	60
6	30.0	485	485	20.0	10.0	60
7	30.0	485	485	20.0	10.0	60
8	30.0[2]	485	485	20.0	10.0	60
Control	0.0	500	500	20.0	10.0	86

[1] Jalapeno peppers were obtained from eight different sources (Table 3).

[2] 9 grams dried pepper added to 21 grams H_2O (as recommended by manufacturer–3/10 replacement ratio).

Table 4. Process cheese formulations. (continued)

Series II - Same Pepper Sample - Varying Pepper Concentrations (0-5%)

Pepper Sample No.[1]	Grams Pepper	Grams Aged Cheddar (16 months)	Grams Young Cheddar (5 months)	Sodium Citrate (grams)	Salt (grams)	Water (mls)
7	10.0	495	495	20.0	10.0	77
7	20.0	490	490	20.0	10.0	69
7	30.0	485	485	20.0	10.0	60
7	40.0	480	480	20.0	10.0	51
7	50.0	475	475	20.0	10.0	43
Control	0.0	500	500	20.0	10.0	86

[1] Water levels in process cheese adjusted on basis of 87% H_2O content of peppers added.

Samples of uniform size were held at room temperature for 30 minutes then presented to the 5 selected panelists. Samples were in a series of disposable paper cups and eaten with the aid of toothpicks.

The taste panelists were supplied with a reference sample (R), a randomized series of 3 coded cheese samples, and a copy of the multiple comparison test questionnaire (appendix, page 63) adapted from Larmond (23). The reference sample (R) was a duplicate of one of the 3 coded samples, and thus served not only as a reference of comparison for the panelists to use, but also was an internal standard or check on the accuracy of the panel. The other samples in the set represented either two different pepper formulation levels, or were peppers obtained from different sources (Table 3).

The questionnaire (appendix, page 63) choices (descriptive terms) selected by the panel members were converted to numerical scores as shown in Table 5. Panelists were asked if the sample was equal to, hotter than, or milder than the reference sample. Thus if a panelist indicated that one of the three coded samples was "equal to reference", this response was given a numerical score of 5, "hotter" was a score greater than 5, and "milder" was a score less than 5.

STATISTICAL METHODS

As a means of comparing the two analytical methods used to determine capsaicin with the sensory estimation of pepper pungency,

Table 5. Descriptive terms used in multiple comparison taste panel questionnaire and corresponding numerical values.

Descriptive Term	Numerical Value
Extremely Hotter Than Reference	9
Much Hotter Than Reference	8
Moderately Hotter Than Reference	7
Slightly Hotter Than Reference	6
Equal To Reference	5
Slightly Milder Than Reference	4
Moderately Milder Than Reference	3
Much Milder Than Reference	2
Extremely Milder Than Reference	1

correlation coefficients were determined. In addition, a correlation coefficient was determined to compare the two analytical capsaicin methods. Regression equations were determined so as to convert data from either of the two chemical capsaicin methods into organoleptic scores (Scoville values).

An analysis of variance (ANOVA) was conducted using the numerical scores obtained from cheese taste panel studies. Mean scores were compared using the Duncan Multiple Range Test for variable score to determine differences which were statistically significant.

The entire statistical analysis outlined above was conducted using the computer and services of the Kansas State University computer center. The correlation, regression, and ANOVA programs were developed by and used on release from the SAS Institute Inc. (P. O. Box 10066, Raleigh, N. C. 27605).

RESULTS AND DISCUSSION

PRELIMINARY WORK

Preliminary trials were conducted using the Scoville method of pungency determination for the purpose of selecting the five taste panel members to be used in this study. Panel members were selected on the basis of their repeatability and sensitivity to pepper pungency. Potential panelists who were either highly sensitive or insensitive to pungency were eliminated from consideration.

It was found during these trials that sensitivity and reproducibility of taste panelists was improved by using a 3% dextrose solution to dilute the pepper extracts, as recommended by Suzuki et al. (46), in place of 5% sucrose as specified in the Quartermaster (1) and ASTA methods (36). Adding ethanol to diluted pepper extracts used in taste panel studies in order to attain the same level of ethanol as in the most concentrated extract was also found to be desirable when 5% sucrose was used. However, because good taste panel results were obtained using 3% dextrose, this modification of the standard Scoville method was not adopted for this study. Sipping warm water as recommended in the ASTA procedure (but not in the Quartermaster) also was found to improve results.

The preparation of several trial batches of process cheese with jalapeno peppers demonstrated that 1:1 ratio of aged and young

Cheddar cheese produced a product of suitable body and texture when sodium citrate was used as the emulsifying agent.

Samples for GLC analysis were initially prepared from both tinctures and oleoresins. It was decided to use tincture samples for this study because they produced cleaner chromatograms and more reproducible results. However, the oleoresin procedure is included in the appendix, page 64.

PUNGENCY (SCOVILLE VALUE) DETERMINATION BY SENSORY ANALYSIS

Results of the organoleptic pungency analysis are shown in Table 6. The average Scoville value for all peppers was 9,225. Individual samples ranged from a high of 15,000 to a low of 3,400 Scoville units. Values obtained from our panels appeared to be somewhat lower than those reported by other researchers (46, 51). In studies using pure capsaicin, the majority of our five panel members were able to detect capsaicin levels of one part in 7.5 million. This compares with threshold pungency levels of one part in 15 million and 16.1 million reported by Suzuki (46) and Todd (51) respectively.

Fresh and dried samples produced higher Scoville values than peppers processed by freezing, fermentation, or canning (Table 6).

Table 6. Pungency of jalapeno peppers and pure capsaicin as determined by sensory analysis.

Sample Number[1]	Sample	Pungency (Scoville Units)
4	Fresh	15,000
2	Fresh	14,500
8	Dried	12,200
3	Fresh	8,800
1	Fresh	8,100
5	Blanched/frozen	7,900
7	Fermented	3,900
6	Canned	3,400
Average		9,225
	Pure Capsaicin[2]	7,500,000

[1] Jalapeno peppers were obtained from eight different sources (Table 3).

[2] Obtained from the Sigma Chemical Co., St. Louis, Mo.

CAPSAICIN DETERMINATION BY ULTRA-VIOLET SPECTROPHOTOMETRY

The results of the ultra-violet spectrophotometric determination of capsaicin are summarized in Table 7. The average concentration of capsaicin was 1.01 mg/1.0000 g dried pepper. Values obtained for the eight pepper samples ranged from a high of 1.36 to a low of 0.53 mg/g dried pepper. These values compare favorably with those previously reported. For example, Weisenfelder et al. (58) reported an average capsaicin concentration of 1.12 mg/1.00 g dried pepper. The precision using duplicate samples (low value divided by high value) ranged from 88.2% to 98.2% and averaged 92.49%. As in the organoleptic analysis, fresh and dried samples produced the highest values.

CAPSAICIN DETERMINATION BY GAS-LIQUID CHROMATOGRAPHY

Results of the gas-liquid chromatographic analysis for capsaicin are shown in Table 8. Capsaicin values obtained for the eight pepper samples ranged from .216% to .032% (w/w) of the dry sample weight. The average for all of the samples tested was .111%. An average of .111% would be equivalent to 1.11 mg capsaicin/g dry pepper, which compares to the average of 1.01 mg capsaicin/g obtained by uv absorbance. A dried sample produced the highest value followed by fresh, blanched/frozen, fermented/pickled, and pickled/canned samples, in that order (Table 8).

Table 7. Capsaicin in jalapeno peppers as determined by ultra-violet spectrophotometric analysis.

Sample Number[1]	Type	Average Absorbance @ 280 nm	Mg Capsaicin per g of Dried Pepper
2	Fresh	.0890	1.36
8	Dried	.0875	1.33
4	Fresh	.0840	1.28
3	Fresh	.0725	1.09
1	Fresh	.0615	0.91
5	Blanched/Frozen	.0615	0.91
7	Fermented/Pickled	.0465	0.67
6	Canned	.0380	0.53
Average			1.01

[1] Jalapeno peppers were obtained from eight different sources (Table 3).

Table 8. Capsaicin in jalapeno peppers as determined by gas-liquid chromatography.

Sample Number[1]	Type	% (w/w) Capsaicin In Dried Sample	Mg Capsaicin per g of Dried Pepper
8	Dried	.216	2.16
4	Fresh	.184	1.84
2	Fresh	.180	1.80
3	Fresh	.113	1.13
1	Fresh	.099	.99
5	Blanched/Frozen	.034	.34
7	Fermented/Pickled	.033	.33
6	Canned	.032	.32
Average		.111	1.11

[1] Jalapeno peppers were obtained from eight different sources (Table 3).

COMPARISON OF METHODS USED FOR PUNGENCY AND CAPSAICIN DETERMINATIONS

Table 9 is a comparison of the three methods used for evaluating pungency. Values for each sample are shown as mg/g of dried sample and as percent capsaicin in dried pepper. The percent capsaicin figures based on Scoville values were obtained by dividing the Scoville values (Table 6) by the capsaicin threshold level determined for our taste panel; 7.5 million.

The best agreement between the three methods was for sample number 3 for which highest and lowest estimates of capsaicin differed by only .008%. The poorest agreement was found in samples number 1 and 8 which had percent capsaicin values differing by .072 and .083, respectively. The average range of difference between the three methods for the eight samples was .045 percent capsaicin.

In general, the three methods of capsaicin (pungency) analysis showed greater agreement with mild samples than with hotter samples. For example, all three methods ranked the five mildest samples (pungency rank 1 - 5) in the same order while mixing the order of the three hottest samples (pungency rank 6 - 8).

Correlation coefficients were determined as a means of comparing the methods of analysis. Good correlations were obtained

Table 9. Comparison of methods used to evaluate pungency[1] and capsaicin[2].

Sample Number[3]	Pungency Rank[4]	Scoville		U-V Spectrophotometry			Gas-Liquid Chromatography		
		mg Capsaicin[5]/g	% Capsaicin	Pungency Rank	mg Capsaicin/g	% Capsaicin	Pungency Rank	mg Capsaicin/g	% Capsaicin
6	1	.45	.045	1	.53	.053	1	.32	.032
7	2	.52	.052	2	.67	.067	2	.33	.033
5	3	1.05	.105	3	.91	.091	3	.34	.034
1	4	1.08	.108	4	.91	.091	4	.99	.099
3	5	1.17	.177	5	1.09	.109	5	1.13	.133
8	6	1.63	.163	7*	1.33	.133	8*	2.16	.216
2	7	1.93	.193	8*	1.36	.136	6*	1.80	.180
4	8	2.00	.200	6*	1.28	.128	7*	1.84	.184
Average		1.23	.123		1.01	.101		1.11	.111

1 Pungency expressed in terms of capsaicin content.

2 All data based on weight of dried sample.

3 Jalapeno peppers were obtained from eight different sources (Table 3).

4 Pungency rank (1=mildest, 8=hottest).

5 Calculated based on ratio of Scoville values to the taste panel threshold for pure capsaicin; 7,500,000.

* Ranking not in agreement with rank assigned by Scoville evaluation.

as can be seen in Table 10. The best correlation, .96, was between the Scoville method and the ultra-violet spectrophotometric method. In contrast, the mean value for % capsaicin in samples analyzed by the Scoville method (.123%) compared more favorably with the mean for GLC values (.111%). This lack of agreement between mean values and correlation coefficients could possibly be explained by the fact that although sample values obtained by uv spectrophotometry were usually lower than those obtained by the Scoville method they generally differed from the corresponding Scoville values by less than those obtained by GLC. GLC values for % capsaicin in contrast, had a wider range of difference when compared to the corresponding values obtained from the Scoville method even though the overall means compared more favorably.

Regression equations (Table 11) were developed so as to relate the two quantitative capsaicin methods, uv spectrophotometry and GLC, to the sensory determination of pungency by the Scoville method.

FAT, MOISTURE, AND pH ANALYSIS OF CHEESE

The results of the fat, moisture, and pH analysis of the natural and process cheese used in this study are shown in Table 12.

The moisture content of the 12 batches of process cheese varied from 33.4% to 36.1% averaging 34.8%. All of the samples of process cheese were within the U.S. Government's legal limit of 41% moisture

Table 10. Correlations for three methods of pungency[1] analysis.

Methods Compared	Correlation
Scoville vs. Spectrophotometry	.96
Scoville vs. GLC	.90
Spectrophotometry vs. GLC	.92

[1] Pungency expressed in terms of capsaicin content. Scoville values were divided by 7,500,000 to obtain a sensory estimate of capsaicin.

Table 11. Mathematical relationship between analytical methods as determined by regression analysis.

Scoville Units = (Absorbance @280 nm) x 221981.88 − 5772.65
Scoville Units = (% Capsaicin by GLC) x 52790.07 + 3345.50

Table 12. Fat, moisture, and pH analysis of natural process cheese.

Cheese Description	% H_2O	pH	% Fat
Aged Cheddar (14 months) used in Series I processing	34.6	5.31	31.5
Young Cheddar (5 months) used in Series I processing	34.1	5.32	32.7
Series I - Control (No Peppers)	36.1	5.67	30.0
Series I - 3% pepper source No. 3	34.7	5.66	31.5
Series I - 3% pepper source No. 5	33.9	5.65	31.3
Series I - 3% pepper source No. 6	34.4	5.50	31.0
Series I - 3% pepper source No. 7	34.5	5.49	32.0
Series I - 3% pepper source No. 8	36.0	5.65	30.5
Aged Cheddar (16 months) used in Series II processing	31.5	5.40	35.5
Young Cheddar (5 months) used in Series II processing	34.1	5.32	32.7
Series II - Control (No Peppers)	34.4	5.79	32.3
Series II - 1% pepper source No. 7	33.4	5.68	31.8
Series II - 2% pepper source No. 7	33.8	5.67	32.5
Series II - 3% pepper source No. 7	36.1	5.66	31.0
Series II - 4% pepper source No. 7	34.1	5.66	32.0
Series II - 5% pepper source No. 7	35.7	5.64	31.6

as specified by the U. S. Government (53) for process cheese with fruits and vegetables.

The fat content of the process cheese samples ranged from 30.0% to 32.5%. Only the Series I control sample, which was not used in taste panel studies, fell below the legal minimum of 30.5% fat as designated in the Federal Code of Regulations (53). The average fat content of all process cheese samples was 31.46%.

The pH of process cheese samples averaged 5.64. As can be determined from the results of Series II samples, the addition of increasingly larger amounts of pickled/fermented pepper in the blend progressively reduced the pH of the cheese. All of the samples conformed to the federal specifications which states that the pH of process cheese shall be not less than 5.3 (53).

TASTE PANEL EVALUATION OF PROCESS CHEESE WITH JALAPENO PEPPERS

The results of taste panel evaluations of process cheese with jalapeno peppers samples are shown in Tables 13 and 17 (analysis of variance) and Tables 14, 15, 18, and 19 (treatment mean separations).

To check the accuracy of the panelists, reference duplicates were included in all tests of both Series I and Series II. These duplicates were not found to differ significantly from the corresponding references (Tables 5, 14, and 18).

Table 13. Analysis of variance of taste panel data pertaining to process cheese with differing pepper samples at 3% level. Series I.

Variance Source	DF	M. S.	F
Judge	4	5.53	5.11[1]
Reference Type	2	2.74	2.54
Sample Type	5	21.14	19.56[1]
Reference x Sample	10	1.01	.94
Residual Error	68	1.08	

[1] Significant F value at alpha level .01.

Table 14. Effect of pepper source on the sensory estimation of the pungency of process cheese with 3% jalapeno peppers.

Reference Scoville Value[4]	Taste Panel Means[1, 2, 3]				
	Sample Scoville Value[4] (Sample No.)[4]				
(Sample No.)[4]	3,400 (6)	3,900 (7)	7,900 (5)	8,800 (3)	12,200 (8)
3,900 (7)	3.4^{a}	4.8^{b}	5.4^{bc}	5.8^{bc}	6.6^{c}
7,900 (5)	3.2^{a}	4.0^{ab}	5.0^{b}	4.0^{ab}	7.0^{c}
8,800 (3)	3.2^{a}	4.0^{ab}	5.0^{b}	4.4^{b}	7.0^{c}

1 Means in the same horizontal row differing by more than 1.31 are significantly different.

2 Means in the same horizontal row not followed by the same letter are significantly different at the .05 level as determined by Duncan's Multiple Range Test for Variable Score.

3 Means calculated from data collected using a multiple comparison questionnaire (Appendix, page 63 and Table 5).

4 Scoville value of the jalapeno peppers was determined prior to formulation of process cheese. Peppers were obtained from various sources (Table 3) so as to obtain a range of pungency.

Table 15. Mean separation of combined taste panel data for process cheese with peppers from different sources added at the 3% level.

Scoville Value (Sample No.)[3]	Means[1, 2]
3,400 (6)	3.266[a]
3,900 (7)	4.266[b]
7,900 (5)	5.113[c]
8,800 (3)	4.733[bc]
12,200 (8)	6.866[d]

[1] Means are for combined panel data.

[2] Means not followed by the same letter differ significantly from one another at the .05 level as determined by Duncan's Multiple Range Test for Variable Score.

[3] See Table 3.

Table 16. Effect of range in Scoville units (SU) between process cheese formulations on recognition of pungency differences by taste panelists.

Sample Comparisons	Sample Pungencies	Range in SU
7 vs 6	3,900 vs 3,400	500[1]
3 vs 5	8,800 vs 7,900	900
5 vs 7	7,900 vs 3,900	4,000[1]
8 vs 5	12,200 vs 7,900	4,300[1]
3 vs 7	8,800 vs 3,900	4,900
3 vs 6	8,800 vs 3,400	5,400[1]
8 vs 5	12,200 vs 7,900	8,300[1]
8 vs 6	12,200 vs 3,400	8,800[1]

[1] Comparison was statistically significant at alpha level .05.

Table 17. Analysis of variance of taste panel data pertaining to process cheese with the same pepper sample at differing levels.

Series II.

Variance Source	DF	M.S.	F
Judge	4	2.88	2.91
Reference Type	2	66.03	66.66[1]
Sample Type	5	19.55	19.73[1]
Reference x Sample	10	3.01	3.05[1]
Residual Error	77	0.99	

[1] Significant F value at alpha level .01.

Table 18. Effect of pepper formulation level on the sensory estimation of the pungency of process cheese with 1 to 5% jalapeno peppers.

Percent pepper in reference	Taste panel means[1, 2, 3] Percent pepper[4] in sample				
	1%	2%	3%	4%	5%
1%	4.8[a]	6.0[ab]	6.0[ab]	6.8[ab]	8.6[c]
3%	4.0[a]	3.6[a]	4.6[ab]	5.7[b]	7.0[c]
5%	2.0[a]	2.4[a]	3.0[a]	2.4[a]	5.0[b]

1 Means in the same horizontal row differing by more than 1.25 are significantly different.

2 Means in the same horizontal row not followed by the same letter are significantly different at the .05 level as determined by Duncan's Multiple Range Test for variable score.

3 Means calculated from data collected using a multiple comparison questionnaire (Appendix, page 63 and Table 5).

4 Jalapeno peppers used in this series of formulations had a Scoville value of 3,900 and were obtained from source 1 (Table 3).

Table 19. Mean separation of combined taste panel data for process cheese with 1 to 5% jalapeno peppers.

Percent pepper in sample[1]	Mean[2, 3]
1%	3.600[a]
2%	4.000[ab]
3%	4.642[bc]
4%	5.150[c]
5%	6.866[d]

1 Jalapeno peppers used in this series of formulations had a Scoville value of 3,900 and were obtained from source 1 (Table 3).

2 Means are for combined taste panel data.

3 Means not followed by the same letter differ significantly from each other at the .05 level as determined by Duncan's Multiple Range Test for Variable Score.

For Series I samples, which utilized different pepper samples at a constant pepper concentration, a significant F value was obtained for sample type (Table 13) indicating that the pungency of a sample influenced the panelists rating of that sample. An illustration of this can be seen in Table 14 where the means of the hottest (No. 8) and mildest (No. 6) samples were significantly different from each other and in several instances were significantly different from samples of intermediate pungencies (Nos. 3, 5, 1). In addition the significant F value obtained for judges indicates that not all judges ranked their samples equally (eg; some judges rankings were consistently different from others).

Sample means analyzed using Duncan's Multiple Range Test for variable score (Table 15) seem to indicate that panelists were more successful in identifying the hottest sample (No. 8) and the mildest sample (No. 6) than those with intermediate pungencies (No. 3, 5, 7).

Comparing the cheese taste panel results with the Scoville values obtained for the pepper samples used (Table 16), it can be seen that in only one case (samples 3 vs 7) was a difference of more than 900 Scoville units not found to be statistically significant. In addition, taste panelists appeared to be more successful when comparing mild samples (6 vs 7) and in identifying the hottest sample (8 vs all others) than in separating samples with a medium range of pungencies (combinations with samples 3, 5, 7).

Statistical analysis of cheese samples in Series II (Table 17), which contained the same pepper sample blended at different concentrations, resulted in significant F values for reference type, sample type, and interaction of sample reference. Mean scores shown in Table 18 indicate that the panelists had better success ranking pepper concentrations in cheese samples when milder references were used. In fact, when a 5% reference was used no significant differences were obtained between any of the samples containing less than 5% peppers. Panel members successfully identified the hottest sample (5% pepper) but had considerable difficulty ranking samples with reduced pepper content (1%, 2%, 3%, 4%). In addition, the reference used was found to significantly influence the rating of cheese samples by progressively decreasing the mean of each sample as the concentration of pepper in the reference was increased.

Mean separation of combined taste panel data (Table 19) indicate that differences of 1% in the pepper concentration were not found to be statistically significant except in the case of 5% pepper samples. In contrast, all differences of 2% or more were found to differ significantly.

SUMMARY AND CONCLUSIONS

One of the objectives of this study was to compare several methods of determining pungency in jalapeno peppers and to investigate their potential for use by food processors. Three methods were studied. The oldest, yet simplest and cheapest, was the organoleptic Scoville method. This method was found to correlate very closely with more complicated chemical determinations but required a trained taste panel to be used successfully. Of the two chemical methods studied, the simplest and the one with the closest correlation to the organoleptic method was the ultra-violet spectrophotometric method. This method has the advantages of a very simple sample preparation and analytical procedure but was found to be somewhat slow and required an expensive ultra-violet spectrophotometer. The gas-liquid chromatography method was complicated by sample preparation and required a highly trained technician to perform the analysis. In addition, the GLC method required the most expensive equipment and chemicals. The mean capsaicin value obtained from the GLC analysis, .11%, compared quite closely to the .12% calculated from organoleptic results.

Since all of three methods of pungency determination correlated closely, the one preferred method for a food processor is a choice that would be dictated by individual circumstances. If the obvious

problems in selecting, training and retaining taste panel members could be overcome, the Scoville method would probably be preferred because of its simplicity and low cost. On the other hand, if a uv-spectrophotometer were available, the uv-spectrophotometric method would be a likely choice since it produces good results although not as quickly as the Scoville method. In addition, the uv-spectrophotometric method does not require a high degree of technical skill on the part of the person performing the analysis. The GLC method, because of its high cost and degree of technical skill required to prepare samples and operate the gas chromatograph, would probably not be preferred over the other two methods.

A second objective of this study was to determine what differences in pungency might be found in pepper samples obtained from different sources.

The 8 samples studied ranged in value from 3,400 to 15,000 Scoville units. Although these Scoville values were somewhat lower than expected, calculation of % capsaicin by dividing Scoville values by 7.5 million (the minimum taste panel threshold level determined for pure capsaicin) produced a range of .045 to .200% capsaicin which is similar to those reported by other researchers.

Examination of the samples by GLC and uv-spectrophotometry produced ranges of .032 to .216% capsaicin and .053 to .136 % capsaicin respectively.

The average capsaicin concentration of all samples tested by the three methods was .112%. Fresh samples and dried samples were generally found to be more pungent than more highly processed samples such as canned, pickled, blanched, frozen, and fermented peppers.

A third objective was to determine to what extent variations in the pungency of peppers were detectable in process cheese with jalapeno peppers.

In a series of cheeses containing 3% jalapeno peppers, it was found that differences in the Scoville values (SU) of peppers as small as 500 were detectable when mild samples (3,400 and 3,900 SU) were compared. In addition, taste panelists were generally successful in identifying the hottest (12,200 SU) and mildest (3,400 SU) samples. However, when samples of intermediate pungency (7,900 and 8,800 SU) were compared, taste panelists failed to distinguish these from each other and in some cases from milder samples.

In another series of cheeses in which peppers from the same source were blended in the cheese at levels varying from 1 to 5% in 1% increments, differences of 1% in the pepper level were not found to be statistically significant except in the case of 5% samples compared to 4% samples. All differences of 2% or more in the pepper blend were found to be significantly different.

APPENDIX

SCOVILLE PUNGENCY "HEAT" TEST

Explanation: The following test is designed to determine the level of pungency in hot pepper (Capsicum annuum) samples by using taste panel members to determine the dilution level at which they first experience the "hot" or "burning" sensation commonly associated with jalapeno peppers. The panel members will be required to taste and swallow 5 ml portions of a 3% dextrose in distilled water solution which contains a very small amount of pepper extract in ethanol.

Instructions: Beginning with sample number 1, quickly transfer the entire 5 ml sample to the back of the mouth and swallow immediately. Note whether a definite sensation of "hotness" or "burning" occurs at the base of tongue and throat. If no "hotness" is detected, wait approximately five minutes and repeat the procedure with succeeding samples. Sip or rinse mouth with warm water between each sample.

Note: After a definite "hotness" is recorded, it is not necessary to proceed with further samples.

Scoville Pungency "Heat" Test (Continued)

Sample Code _____ Name of Panelist _____

Date _____

Sample No.	"hotness" detected	No "hotness" detected
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____

Name _____ Date _____

QUESTIONNAIRE:

You are receiving samples of jalapeno pepper cheese to compare for pungency (hotness). You have been given a reference sample marked R, to which to compare each sample. Test each sample; show whether it is more pungent (hotter) comparable to, or less pungent (milder) than the reference. Then mark the amount of difference which exists.

Sample Number	_____	_____	_____
Hotter than R	_____	_____	_____
Equal to R	_____	_____	_____
Milder than R	_____	_____	_____

AMOUNT OF DIFFERENCE:

None	_____	_____	_____
Slight	_____	_____	_____
Moderate	_____	_____	_____
Much	_____	_____	_____
Extreme	_____	_____	_____

COMMENTS:

Any comments you may have about the flavor, texture, color, etc., may be made here:

PREPARATION OF OLEORESIN SAMPLES FOR ANALYSIS BY GLC

Oleoresins were prepared by concentrating filtered alcoholic extracts of dried, ground pepper samples in a rotary evaporator at 55 C to a volume of approximately 10 ml. The concentrated extract was then transferred to a 55 C water bath where carbon dioxide (CO_2) gas was bubbled through the sample until a gummy residue (oleoresin) remained. A carefully weighed amount (.1 – .2g) of the oleoresin was then combined with 5 ml of THF. Further preparation for GLC analysis was identical to that used for tinctures (page 25).

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VARIATION IN PEPPER PUNGENCY AS A FACTOR IN THE QUALITY OF PROCESS CHEESE WITH JALAPENO PEPPERS

by

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B. S., Kansas State University, 1971

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1979

Abstract

Victor Lee Doperalski, Master of Science, 1979

Major: Food Science, Department of Animal Science and Industry

Title of Thesis: Variation in Pepper Pungency as a Factor in the Quality of Process Cheese with Jalapeno Peppers

Directed by: Dr. James T. Marshall
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Pages in Thesis: 71 Words in Abstract: 296

ABSTRACT

Jalapeno peppers from eight different sources were analyzed for pungency (hotness) by organoleptic evaluation, ultra-violet spectrophotometry and gas-liquid chromatography. Samples were found to differ by 11,600 Scoville units (SU), .83 mg capsaicin/g, and .18% capsaicin respectively. Correlations between these methods were found to be above .902 in all cases. The best correlation, .96, was obtained between the organoleptic and ultra-violet spectrophotometric methods. Five of the eight pepper samples were used to make process cheese with jalapeno peppers. The cheese samples were evaluated for hotness by a five member taste panel who compared each sample to a reference sample. Evaluations were converted to numerical scores ranging from 1 = extremely mild, to 9 = extremely hot, when compared to the reference. Means obtained from the taste panel scores were analyzed for statistical differences. In one series

of cheese samples, where different pepper samples were blended at the 3% level in the cheese, panelists could recognize differences in the pungency in 6 out of 8 comparisons. A difference of 500 Scoville units (SU) between pepper lots was detected between cheeses made from milder peppers (3,400 and 3,900 SU), while for peppers of intermediate pungency (7,900 and 8,800 SU) a difference of 900 SU was not detected. In all cases, panelists identified cheese made with the pepper sample of highest pungency (12,200 SU). In another series of cheese samples in which peppers from the same source were blended in the cheese at levels ranging from 1 to 5% in 1% increments, differences in the pepper level of 1% between samples were not found to be statistically significant except when a 4% sample was compared to a 5% sample. All differences of 2% or more in the pepper level in cheese samples were found to be significantly different.